MONTHLY PROGRESS REPORTS

Period Covered

November 1-30, 1985

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RESEARCH CENTER

RICHMOND, VIRGINIA

MONTHLY PROGRESS REPORTS

Period Covered

November 1-30, 1985

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Date Issued

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Product Development

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CHARGE NUMBER: 1990

PROJECT TITLE: BLEND DEVELOPMENT

PROJECT LEADER: C. M. MOOGALIAN

PERIOD COVERED: NOVEMBER, 1985

I. Merit Select

Nine models have recently been made in semiworks:

1. Merit (Control)
2. Merit with Special Oriental Expanded
3. Merit with Special Bright Expanded (12 months duration)
4. Merit with Special Bright Expanded (24 months duration)
5. Same as #4, but without AC
6. Marlboro (Control)
7. Marlboro with Special Bright Expanded (12 months duration)
8. Marlboro with Special Bright Expanded (24 months duration)
9. Same as #8, but without AC

The special expanded brights are primarily the thin grades from the DBC line. Also, 100% cigarettes of the special component's used in this study have been made. Subjective and analytical evaluations expected to be completed by mid-December.

II. PM Super Lights

Models will be made for subjective evaluations by early December.

The blend for this project was formulated from U.S. tobacco inventories with the cooperation of the Leaf Department. Special Bright expansion (bodied tobaccos) is included in the formulation.

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/adv

C. M. Moogalian

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CHARGE NUMBER: 2100

PROJECT TITLE: NEW PRODUCT INNOVATIONS

PROJECT LEADER: H. V. LANZILLOTTI H.V.L./adv

PERIOD COVERED: NOVEMBER, 1985

I. Project Vanguard

Cooperative program initiated with Physical Research, Chemical Research, Flavor Development and Product Evaluation divisions. Program objectives are as follows:

Short Range: Develop "cold swept" device preferred to Favor.

Medium Range: Develop device which delivers smoke flavored aerosol. May be attached to desk mounted power supply.

Long Range: Develop device which looks and feels like a cigarette and gives similar satisfaction.

Status:

Short Range: "Cold Swept" concepts being handmade and tested for feasibility. Several models being used for flavor screening. Flavor release from PVA (Flavor Dot technology) as well as encapsulation of water and flavors being investigated for applicability.

Medium Range: Flavor and Blend testing continues for hand held electric cigarettes. Blends with higher humectant levels produced less visible aerosol.

Long Range: Subjective tests designed for piezoelectrically and ultrasonically generated aerosols.

II. Flavor Dot

Objective:

To develop a filter flavor release system, with or without a unique end appearance.

Status:

Series of PVA/menthol formulations being tested for menthol release. Homologous series of flavor components being prepared by Flavor Development. Test initiated to determine if components other than menthol can be released from PVA and if so, what are pertinent chemical properties.

III. Project Premium

Objective:

Develop a cigarette which will be perceived by the consumer as a premium product both in appearance and taste.

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Project Premium Cont'd

Status:

Machine made models requested incorporating three blend types with and without special flavors. Handmade models to demonstrate appearance of various combinations of tipping and cigarette papers being assembled for Focus Group testing.

IV. Low Tar Full Flavor - Dual Rod Program

Objective:

To develop a cigarette product with dual-rod systems for enhanced taste/impact and unique flavor delivery profiles.

Status:

Models requested for subjective evaluation of combinations of six blend types. Each model is designed to be in 5-6mg delivery range. Smoke delivery data from full flavor models being evaluated to determine effect of dual rod concept on puff by puff delivery profile.

V. Encapsulation

Objective:

Adapt state of the art encapsulation technology for use in new or improved products.

Status:

Flavor Release: Laboratory experiments in progress to develop frangible gelatin microcapsules containing flavors for release by "scratch and sniff" technique.

Water Release: Pack OV studies in progress for encapsulated water coated on pack foil innerwrap.

Experiments being conducted to evaluate feasibility of water release from sealed microporous polypropylene packets containing water absorbed on blotter board.

VI. Instrumentation Services

Objective:

Evaluation of commercially available equipment and development of test procedures.

Status:

Program conducted with Process Engineering to improve process for determination of RTD of Barclay cigarettes using PM designed "Labyrinth" cigarette holders. Four models of cigarette insertion devices produced. Preferred model will be submitted for evaluation by FTC.

Evaluation in progress of Filtrona automatic hardness tester and automatic filter test station.

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CHARGE NUMBER: 2105

PROJECT TITLE: FILTER DEVELOPMENT

PROJECT LEADER: W. A. NICHOLS

PERIOD COVERED: NOVEMBER, 1985

I. Filter Development - FML (W. Nichols)

Objective:

To develop the technology to manufacture filters from FML polypropylene tow.

Status:

Significant reduction in filter pressure drop coefficient of variation has been achieved. Sample tow produced with new equipment has a RTD C.V. of 3.5% to 5.5%. Previous samples yielded RTD C.V. of 6% to 10%. Improvement in RTD control is attributable to modifications made to fibrillation and crimping.

Variation of tow band width and edge quality causes lower plugmaker efficiency. Initial analysis of the problem suggests that tow band tension changes between the fibrillator and crimper. Testing has verified changes in tension and a tension control device is being evaluated.

Cast film samples were produced at a vendor's laboratory for evaluation. Cast film is more uniform in thickness than blown film, which is currently used for tow production.

Based on the progress made during the past year, the FML Board of Directors approved continuation of development and the 1986 Budget.

Plans:

Reduce Tow Wastage to <5%	June, 1986
Prepare samples for Additional Market Evaluation	Continuous

II. Tobacco Extrusion (R. Thesing)

Objective:

Develop subjectively acceptable extruded models.

Status:

Internal panel testing was postponed with the recent low density (450 mg) models due to excessive hot rod collapse. Samples were re-made to a 500 mg weight specification. Hot collapse should be minimized. Rods will be machine tipped and packed the week of 12/2/85. Samples will be available for internal testing the week of 12/9/85. Both the charcoal on tow/CA filter and the Dunhill Lts/CA filter attached to the extruded rod, will be tested.

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Tobacco Extrusion Cont'd:

450 mg rods made with Avicel will be tested for firmness while smoking. Bruce Lossee continues his evaluation of mass burn rate of various porosity extruded rod models.

Plans:

Internal Panel Results	January, 1986
Improve subjectives & hot rod collapse	Continuous

III. Menthol Application (G. Patron)

Objective:

Assist in the introduction of menthol on foil into production.

Status:

Calibrations were performed to determine the required menthol application target level for Players Lights 25 KS and 100s and Cambridge Lights KS and 100s. The target menthol in smoke is 0.60 mg in the field. The estimated total menthol level per cigarette is 4.3 mg for the 85s and 5.2 mg for the 100's. Foil bobbins were mentholated for small sample productions of Cambridge Lights KS (in semiworks) and Players Lights 25 KS (in M/C). Cigarettes produced will be aged for two weeks prior to CI analysis.

The mentholating machine shipped to Louisville was installed and an initial start-up run was completed. A second mentholating machine was installed in R&D for continuing brand development and process testing. This machine will rewind foil bobbins up to a maximum speed of 360 meters per minute (mpm). However, test runs conducted to date were limited to 300 mpm.

Plans:

Assist brand development group in the production of Cambridge Lights KS Menthol	December 1985
Test PM Mentholator at 360 mpm	1st Qtr., 1986
Continue training of Louisville personnel	1st Qtr., 1986

/adv

W.A. Nichols/adv

W. A. Nichols

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CHARGE NUMBER: 4009

PROJECT TITLE: SMOKE STUDIES

PROJECT LEADER: B. L. GOODMAN

PERIOD COVERED: NOVEMBER, 1985

I. Project Slow (J. Hearn)

Objective:

Develop the technology to reduce or mask objectionable odor of sidestream smoke.

Develop a subjectively acceptable cigarette with reduced sidestream visibility.

Status:

Blend and filtration changes yielded full flavor models which were found by the sidestream panel to be more subjectively acceptable than the previous prototype tested on the MC panel in October. The improvements were incorporated into one new model, which had a generally favorable subjective response. It will be resmoked after proper aging.

Low delivery models with three different aftercut flavors were evaluated and one prototype was found to warrant further testing. Prototypes have also been made with charcoal filters. They will be tested on a Richmond only cross Operations panel along with a regular CA model.

Two high citrate wrapper models with about 45% visibility reduction have been made with blend modifications and will be evaluated when analytical data is available.

Plans:

To evaluate menthol models of both the full flavor and low delivery prototypes.

II. Wrapper Modifications (R. Greene)

Objective:

To evaluate modified cigarettes for slow burning characteristics.

Status:

Cigarettes with slow burning characteristics made with low porosity wrappers have been evaluated by Flavor Development with modified blends. An initial screening has identified the better blend and wrapper combinations. Unflavored cigarettes have been requested for additional flavor work.

Several coatings of various phosphate salts have been made to help evaluate the flavor characteristics of these salts while retaining slow burning characteristics.

A Virginia Slims 120 model with an improved filter design has been made and is being analyzed for smoke delivery. Additional designs will be requested once the efficiency of the filter is known.

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III. Computer Applications (R. Greene)

Objective:

To write programs in BASIC and provide user assistance for the directorate at O/C.

Results:

A new command has been added to DELIVERY, the smoke delivery analysis and data-base program. The new command allows the user to create a file which summarizes the titles and codes contained in any number of delivery codes of keywords within this master file, then call the data from the appropriate data file.

IV. New Products (R. Arthur)

Objective:

To determine consumer response to cigarettes with modified tobacco wrappers.

Status:

Kimberly-Clark has sent us sample bobbins of wrappers that contain half paper and half tobacco. Different shades of brown were requested in both a solid and a veined look. The wrappers were made into 11 mg cigarettes for focus group testing. Different tipping paper combinations were also presented to the focus groups. Preliminary results show that there was some interest in a tobacco wrapper, but not in just a brown or tobacco colored regular paper.

/adv

B. Goodman

B. L. Goodman

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CHARGE NUMBER: 4015

PROJECT TITLE: NEW PRODUCT DEVELOPMENT

PROJECT LEADER: W. G. HOUCK, JR.

PERIOD COVERED: NOVEMBER, 1985

I. Project DATA

Objective:

To develop an adjustable tar cigarette product.

Status:

DATA test market of Concord Regular and Menthol 85's is in progress.
Completed making of Merit Select prototypes with different blends.
Made initial prototypes from Concord 100mm Maker.

Plans:

Product Optimization	Ongoing
Process Optimization	Ongoing
100mm Test Market	Feb., 1986
Test merit Select Prototypes	Jan., 1986

II. Project Fresh

Objective:

To develop 12 and 16 mg versions of a free-standing menthol product with special packaging for "freshness" perception.

Status:

Completed initial models at 12mg with four flavor systems on High Lamina blend.
Developing additional models (12 and 16 mg) on new "menthol" blend from Blend Development group.

Plans:

Model Development	Dec. '85/Jan. '86
Consumer Testing	1st Qtr., 1986

III. Players Lights 25's

Objective:

To develop a 10mg tar 85mm and a 12mg tar 100mm cigarette in both Regular and Menthol to be sold as a value entry product with 25 cigarettes per pack.

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Players Lights 25's Cont'd

Status:

Production of both regular and menthol is in progress for national introduction.

Plans:

National Introduction January, 1986

IV. Cambridge Lights 20's

Objective:

To develop a 12mg tar 85 and 100mm value entry product in both Regular and Menthol to compete against Doral.

Status:

Cigarette specifications were finalized with mentholated foil being considered for this product. Test market is scheduled for February 3, 1986 in Denver and Des Moines.

Plans:

Production Start-Up	December 9, 1985
Test Market	February 3, 1986

VI. Project Bristol

Objective:

To develop an 85 and 100 mm generic cigarette in both Regular and Menthol.

Status:

Several models were produced at the request of N.Y. Marketing for field sampling. Nonmenthol cigarettes were produced at two tar levels, 12 and 16 mg, and in both 85 and 100 mm configurations.

Plans:

Low cost blends and the foamed binder technology are being investigated for this program.

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/adv

W. G. Houck

W. G. Houck, Jr.

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CHARGE NUMBER: 5001

PROJECT TITLE: PACKAGING STUDIES

PROJECT LEADER: H. R. DUNAWAY

PERIOD COVERED: NOVEMBER, 1985

Objective:

Assist New Products Directorate in evaluating new packaging concepts and products, and provide technical packaging support to Manufacturing, Manufacturing Services, Engineering, Purchasing, and Quality Assurance.

Status/Plans:

A. Packaging Studies

° Shelf-Life Study: In progress. The feasibility of using Allied Corp.'s Lifelines System as a tool for predicting menthol delivery is being investigated.

° Fermentation Study: Plan to begin testing in December using standard packages with varying pack seal strengths, LAB boxes, plastic cannisters, plastic FTB's and hermetically sealed packages.

° Players Lts. Menthol: Freshly printed packaging materials being used in production were subjectively evaluated by the Flavor Development Odor Panel and found to have an intense solvent odor. GC headspace analysis showed high volume residual solvent peaks.

° VA Slims Lts. 120 Menthol: Samples with a menthol fragrance added to the box varnish are being evaluated.

B. Storage Studies in Progress

° Players Lts. Menthol 85 & 100. Complete 12/86.

° Concord 85 Menthol. Complete 12/19/85.

° Mentholated Foam Binder. Complete 4/86.

C. Storage Studies Completed

° Concord 85 Regular

° B&H Deluxe Lts. Regular

° Marlboro Standardization Run 2

° B&H Deluxe Lts. Menthol

D. Pending

° Evaluation of Alcan gold foil with water base ink. Begin week of 12/9.

° Cambridge Lts. Storage Study. Begin week of 12/9.

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H. R. Dunaway

/adv

H. R. Dunaway

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Process Development

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CHARGE NUMBER: 0307
PROJECT TITLE: Measurements Development
PROJECT LEADER: D. R. Fox
PERIOD COVERED: November, 1985
DATE OF REPORT: December 6, 1985

I. Moisture Measurement

Objective:

To evaluate new or improved methods for the measurement of moisture in filler and cigarettes.

Status:

The ovens in the Materials Evaluation Facility were adjusted to give equal air velocity by installing baffle plates and adjusting the dampers. Monitor testing since the adjustments has been encouraging. A large test to characterize oven variation resulted in an oven-to-oven standard deviation of means of 0.13% (absolute), compared with 0.24% obtained several months ago. Improvements have also been seen in the routine monitors, but additional history will be required to verify it.

Testing resumed on the American Scientific (AS) oven with the arrival of additional dessicators to handle the large capacity (±200 cans) of the oven. Results of late have been more encouraging than initial testing, although we are still struggling with an optimum test design to eliminate time effects in processing the large number of samples. The most recent test showed a within-oven variation comparable to the Precision ovens. However, the AS oven showed side-to-side variation, whereas the Precision ovens generally have shown more front-to-back variation.

Plans:

Monitoring of the MEF ovens will continue, as will the testing of the AS oven. We are also seeking out other types of ovens in use in PM to which we can apply the air velocity measurement method.

II. Materials Evaluation Facility

Objective:

To provide physical testing services (CV, OV, firmness, loose ends, etc.) to groups inside and outside R&D.

Status:

Funding for the new RH-temperature control system has been approved, and a purchase order will be issued. Details of the installation were discussed with Honeywell. The new system will be installed in spring of 1986.

Bids from outside shops have been received for the mechanical construction of a new cigarette weight selector. The bid package was issued by PM Engineering, as they are constructing a unit for QA.

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Plans:

The mechanical contract for the weight selector will be awarded as soon as funding is approved. The electrical design for the instrument will be done by Development Engineering.

III. Miscellaneous

A second Thunder psychrometer was received for evaluation. Tests against the original instrument, the Assmann psychrometer in the CTSD Standards Lab, and equipment in John Crump's lab in the tower indicate that the two Thunder's read reasonably close to one another, but there is a 0.5 degree offset in the temperature readings compared to NBS thermometers. Thunder is being consulted about the problem, because they supposedly calibrate their temperature elements against the NBS.

The Rhodia firmness-while-smoking instrument has arrived and is being set up. A software problem in the HP-86 computer is currently being studied. In addition, steps are in progress to order another commercial FWS instrument from Rupert International. Both units will be studied to develop a standard PM FWS method.

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CHARGE NUMBER: 0400
PROJECT TITLE: Tobacco Properties Applications
PROJECT LEADER: R. S. Mullins Robert S. Mullins
PERIOD COVERED: November 1985
DATE OF REPORT: December 6, 1985

I. Maker Optimization Program

Objective:

Optimize cigarette makers to reduce degradation of filler and improve product quality.

Status:

A second test has confirmed the results reported last month indicating that the position of the combs in relation to the picker significantly affects filler degradation. In this second test, the percentage of 6 mesh filler at the base of the chimney increased from 4.6% to 6.5% as the distance between the picker and the tips of the combs was increased from 9 mm to 18 mm. At the 18 mm distance, providing a clearance between the combs and the carding drum further reduced degradation, increasing the percentage of 6 mesh filler to 8.6%. At the 9 mm distance, enough clearance could not be obtained to significantly affect degradation.

A survey of the position of the combs on the Mark 9 makers in the Operations Center Semiworks found that the position varied significantly from maker to maker, as much as 6.5 mm out of the possible 9 mm. Significant variation was also found from one side of the combs to the other on several makers. A similar survey of several makers in the Manufacturing Center is being planned to determine if such variation is typical there also.

Plans:

Continue investigating the effects of comb to picker and comb to carding drum spacing on filler degradation and cigarette weight variation. Continue development and evaluation of the pneumatic picker.

II. Coal Strength Studies

Objective:

Investigate the factors affecting cigarette coal strength as measured by the existing coal strength test. Investigate alternate methods of measuring coal strength.

Status:

Additional testing has confirmed that dense ends are detrimental to the coal strength of cigarettes. Marlboro 85 mm cigarettes made to a target weight of 980 mg and containing dense ends had a coal failure rate of 45%. Similar cigarettes without dense ends made to a target weight of 960 mg had a failure rate of only 29%. Cigarettes without dense ends made to a target weight of 940 mg had a failure rate of only 36%. Since the density of the cigarettes made to a target of 940 mg is less than the density at any point in the cigarettes containing dense ends, the lower coal strength of the cigarettes with the dense ends cannot be attributed to their "light" middle.

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Plans:

Continue to investigate the effect of dense ends on cigarette coal strength. Investigate alternate methods of measuring coal strength.

III. Low Density Rod Formation

Objective:

In cooperation with Project 1503, develop a continuous process for the production of low density cigarettes.

Status:

Steam was used in place of compressed air on a pneumatic rod forming device to reactivate binder which had been pre-applied to filler by Project 1503. Hand made rods produced using this technique were well bound. Based on these results, the equipment necessary to demonstrate steam reactivation of pre-applied binder in a continuous process is being obtained.

Plans:

Demonstrate continuous formation of a bound tobacco rod using steam reactivation of pre-applied binder. Develop specifications for a prototype continuous process for the production of low density cigarettes.

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CHARGE NUMBER: 1005
PROJECT TITLE: Primary Process Development
PROJECT LEADER: E. G. Craze
DATE OF REPORT: December 6, 1985

TITLE: HAMBRO DRYER (T. Skidmore)

OBJECTIVE: Evaluate the Hambro vibrating fluidized bed dryer for use in drying tobacco materials during primary processing.

STATUS: Tests to compare the performance of the Hambro with that of the Semiworks Adt dryer have been completed. Preliminary results showed no major differences in the size and CV of the dried filler. OV variation (one sigma) of the material exiting the Hambro was greater than that exiting the Adt (0.76 vs. 0.35) but was about the same at the silo exit (0.24 vs. 0.28). Five hundred pounds of cased bright strip was also dried (from 22.8% to 12.0% OV) in the Hambro without major problems.

PLANS: Evaluate the Hambro for use in drying small (100-200 lbs.) blends of cut filler in the Semiworks and continue testing to evaluate the Hambro with strip and stem materials.

TITLE: SMALL SCALE PROCESSING (T. Skidmore)

OBJECTIVE: Develop a small scale process that will provide cigarette characteristics that are fully comparable to those of the full scale process.

STATUS: Analysis of the three most promising process variations for the small scale operation is almost complete. The smoking panel reported subjective differences between the small scale processes and the large scale control process. Additional tests are planned to determine the significance of the subjective differences.

PLANS: Complete the evaluation of the different processes and define the small scale primary processing operation.

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TITLE: MAKE/PACK OPERATIONS (D. Albertson)

OBJECTIVE: Improve the performance and efficiency of the Semiworks Make/Pack operation.

STATUS: New 100-cigarette weight measurement procedures to verify target weights and an abbreviated QA inspection of trays and boxes have been initiated to assure production quality prior to release. A technician will be added to the group to handle the QA requirements in the Make/Pack area and to coordinate the results of QA audits on POL and HTI tests.

PLANS Cigarette processing guidelines will be established for the Make/Pack operation. The guidelines will define the information needed before a request can be processed and the expected results. Emphasis will be placed on educating requesters on the performance and limitations of the Semiworks Make/Pack operation.

E. G. Craze

E. G. Craze

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PROJECT NUMBER: 1307

PROJECT TITLE : Reconstituted Tobacco Development

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: R. G. Uhl

I. IMPROVED SHEET PROPERTIES

A. Objective

Improve the physical characteristics and blend performance of reconstituted sheet.

B. Status

1. Test RL and RCB products made with alternate humectants at Park 500 and the BL Plant were evaluated in blended cigarettes produced at Stockton Street. Based on subjective screening of these cigarettes, a combination PG/glycerin humectant system was implemented in both sheet facilities.

2. Additional tests were conducted at the BL Plant to produce RCB at a lower overall humectant level, i.e., correcting for residual humectants in the class tobacco portion of the feedstock. This material was subjectively acceptable in 100% cigarettes and will be evaluated in blended cigarettes to be produced at Stockton Street in December. If the lower humectant level is implemented for RCB, all sheet materials would be made to a specification of 3% PG/2.5% glycerin on a bone-dry finished sheet basis.

3. The initial ("zero time") Semiworks survivability study of the new sheet materials is in progress. Initial results indicate that 10-20% of the PG is lost during Primary processing. These Semiworks evaluations will be repeated periodically as the hogsheads age (up to one year of warehouse storage) and will include attrition, stability and subjective testing.

C. Plans

1. Support blended cigarette tests with new humectant sheets at Stockton Street.

2. Complete the initial Semiworks evaluation of new humectant sheet products.

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II. SUBJECTIVE MODIFICATION OF RL

A. Objective

Improve or modify the subjective character of RL.

B. Status

1. The second Semiworks survivability study of RL/RCB product produced at Park 500 in March was completed. This material (with RLTC and 150B controls) had been in hogshead warehouse storage for seven months. Results paralleled initial testing (age = 1 month), i.e., the RL/RCB product was equivalent to the controls in terms of pad generation, long shred survivability and maker dust generation.

2. The bench scale continuous reactor (CSTR) installation for precooked flavor is near completion. Equipment checkout and system debugging will begin in early December.

3. Sheet materials produced using pilot/Park 500 baseweb and CEL (including cross-products) have been made into cigarettes and submitted for subjective evaluation to clarify any offset between the facilities. A pilot RL/RCB sheet was also produced using the original CEL cooking technology to determine if this is still a subjectively acceptable product with the present pilot substrate sheet.

C. Plans

1. Start-up and debug the bench CSTR system.

2. Complete evaluation of cross-product sheets.

R. L. Uhl

/deb

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CHARGE NUMBER: 1503
PROJECT TITLE: Modified Smoking Materials
PROJECT LEADER: J. G. Nepomuceno
PERIOD COVERED: November, 1985
DATE OF REPORT: December 9, 1985

I. Tobacco Extrusion

Objective:

To develop a process for extrusion of foamed tobacco articles.

Status:

Dual filters using a combination of CA on the mouth end and various types of tobacco filler on the rod end were made successfully at the Semiworks using a standard dual filter combiner. Extruded rods tipped with these filters are currently being analyzed for tar deliveries prior to testing with in-house panels.

Plans:

Complete subjective evaluation of extruded rod prototypes using in-house panel by the end of December, 1985

II. Foamed Filler Binder

Objective:

To develop a process for applying a subjectively acceptable foamed binder to the tobacco filler in order to improve coal strength, reduce loose ends and allow for weight reduction.

Status:

Marlboro and Players Lights prototypes with 50 mg weight reductions are being tested analytically and subjectively using in-house panels. A binder formulation containing 12.5% licorice/12.5% degraded pectin was added to these models at 0.5% add-on rate.

Plans:

Complete panel testing of above prototypes by January, 1986.

III. Low Density Rods

Objective:

To develop a process for producing low density cigarette rods.

Status:

A process for producing low density cigarettes using tobacco precoated with binders, forming a low density tobacco structure, then reactivating the binders to form a bound structure was outlined. Prototype components are currently being designed and fabricated for evaluation.

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Plans:

Complete evaluation of prototype low density process described above by June, 1986.

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CHARGE NUMBER: 1704
PROJECT TITLE: Supercritical Fluid Processes
PROJECT LEADER: F. V. Utsch
PERIOD COVERED: November, 1985
DATE OF REPORT: December 6, 1985

SUPERCRITICAL FLUID SAMPLING

Objective:

Develop sampling hardware and techniques that will generate accurate and reproducible results from the supercritical fluids laboratory apparatus.

Status:

Calibration of the SCF laboratory system using naphthalene as the standard solute and employing recirculation through the sample loop was started. Initial tests indicate the pump fabricated by Development Engineering performs well. Solubility results for naphthalene in SCF-CO_2 have generally been within 5% of literature values and as close as 1.7%. However, in the region of steepest density gradient (ca. 1200 psig), the results were consistently 15% higher than expected.

Plans:

To continue with naphthalene solubility experiments through a range of temperatures (35°C, 55°C) and pressures (1200 - 4000 psig) in order to determine the system's behavior at minimum and maximum endpoints and at the midrange. Upon completion of the system calibration, attempts will be made to reproduce the results found in patents on tobacco extraction with supercritical CO_2.

SCE FACILITY:

A report is expected from UHDE shortly which will define the performance criteria of the proposed SCF system including "breakpoint" information on system design, complexity, cost, and operational aspects as a function of the different classes of solvents and the operating temperature and pressure limits to be required. The specifications are being discussed with potential users of the system from various divisions within R&D to ensure that the desired system capabilities are provided.

A meeting is scheduled with UHDE/Chemac, Inc. in early December to negotiate the final terms of the contract.

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CHARGE NUMBER : 1801

PROJECT TITLE : Expanded Tobacco Development

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: G. Gellatly

I. CONVEYOR REORDERING

A. Objective

To minimize DIET filler breakage and filling power loss during reordering.

B. Status

DIET reordered in one minute in the pilot plant (24% longs) was compared with MC DIET (8% longs) of the same filling power at 100%, 30% in DBC bright and 15% (MF 03/86) in cigarette. The longer pilot plant DIET resulted in reduced loose ends in cigarettes of the same weight which confirms previous results. The firmness of cigarettes containing pilot plant product in 100% and 30% blends was, however, lower than the respective MC product blends. The 15% blended cigarettes showed no difference of cigarette firmness at the same weight but indicated an improvement of loose ends in the cigarettes containing the longer pilot plant product. Coal strength results are not yet available.

C. Plans

1. Complete the analysis of the single stage reordered product and issue a report on conveyor reordering.

2. Develop design data to support the installation of a production test facility at Cabarrus for conveyor reordering.

II. UNFOLDED STRIP

A. Objective

To evaluate processing and structural characteristics of unfolded strip for improved filler length and cigarette quality.

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B. Status

Unfolded strip was produced for evaluation of the resulting filler in the BTM cigarette and in a foam bonded rod.

Cigarettes containing DIET from unfolded strip filler showed no improvement of loose ends or firmness compared to standard DIET. The longs content of feedstock for this test was 10% abs. greater than that of the control, yet no difference was observed in the longs content of these cigarettes. The reason for this phenomenon may be that DIET processing increases the stiffness and friability of the filler which reduces its survivability through cigarette making.

C. Plans

1. Evaluate unfolded strip filler in a Marlboro blend cigarette.

2. Evaluate unfolded burley strip filler in cigarettes.

3. Study the unfolding of cased and uncased burley strip.

4. Determine if casing unfolded burley strip is detrimental to unfolding.

/deb

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CHARGE NUMBER: 1806

PROJECT TITLE: New Tobacco Processes

PROJECT LEADER: S. R. Wagoner

PERIOD COVERED: November 1-30, 1985

I. STEM TREATMENT

A. Objective

To develop methods of improving the subjective character of bright and burley stems.

B. Status

All stem preparations were completed in the test program designed to identify the mechanism causing the positive subjective modification from treating stems with cellulase. Stem treatments included variations of incubation time (24, 48, 72 hr), incubation temperature (4°C and 50°C), and level of enzyme (0, 8% and deactivated enzyme). Ten pounds of each stem product were prepared.

A pilot plant run, producing an RL sheet from cellulase treated material, was conducted.

C. Plans

Machine-made cigarettes containing 100% of the above treated stem will be produced and submitted for chemical and subjective analyses.

Physical and chemical analyses of the RL sheet will be conducted. Cigarettes will be made from the RL for subjective evaluation.

II. CIGARETTE QUALITY

A. Objective

Evaluate cigarette quality improvements using tobacco materials produced from new technologies.

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B. Status

A test program was initiated to evaluate the performance of tobacco materials that are produced from various new technologies. These materials were whole cut leaf, high tensile RL, improved DIET, unfolded strip, and roll stripped burley. Two blends containing combinations of these materials were produced. The difference between the two was the bright fraction, one being whole cut leaf and one unfolded strip. Control blends, containing materials produced from current production modes, were also made.

C. Plans

Cigarettes will be made from the above blends, and the effect of the test materials on blend and cigarette quality will be determined.

III. FOAMED BINDER

A. Objective

Develop a method to produce a binder for a bonded cigarette rod.

B. Status

Start-up of the pressure vessel has been conducted. Citrus pectin has been successfully degraded, both thermally and enzymatically.

C. Plans

Using the vessel, assist Project 1503 in the development of a binder.

S. R. Wagoner

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Applied Research

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PROJECT CHARGE: 1702

PROJECT TITLE: FILTRATION PHYSICS

PROJECT LEADER: R. W. Dwyer

PERIOD COVERED: November 1 - 30, 1985

MASS BURN RATE (Morgan, Cox, Dwyer)

Experiments are being performed to relate the mass burn rates of smouldering cigarettes with their sidestream visibility values. In order to select candidates for this study, an analysis was performed on data for 89 cigarettes, the data being obtained from the most recent CI report. Multiple regression analysis of this data shows cigarette circumference, rod-paper citrate level, rod-paper porosity, and rod density to be the major factors of those reported which influence static burn rates. Of those four, circumference and rod density were the major factors. Rod density demonstrated the greatest correlation with burn rate as a second order function. This suggests that burn rate will diminish at both low and high densities.

LOW DENSITY RODS (Cox)

A computer simulation of tobacco shred packing is in progress. The equation for the bulk modulus has been modified by incorporating a percolation threshold function. These calculations will provide a check on the validity of the modulus equation in terms of shred length, cross-section, Young's modulus, and bed density.

SIDESTREAM VISIBILITY (Morgan, Cox)

A new cell has been constructed which allows the visibility of the sidestream smoke from seventeen cigarettes to be measured at different heights above the coals. In addition to visibility, the sidestream mass and average particle size can be monitored. Work is in progress to characterize the sidestream of a variety of cigarette brands.

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RWDwyer

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PROJECT CHARGE: 1703

PROJECT TITLE: CIGARETTE MAKING TECHNOLOGY

PROJECT LEADER: A. Robinson

PERIOD COVERED: November 1 - 30, 1985

DATE OF REPORT: November 25, 1985

FLAVORS ADSORPTION STUDIES (A. Robinson, V. P. Henderson)

Our contact-angle goniometer temperature stage and temperature controlled application syringe have been received. The initial results suggest that the adsorption and absorption behavior of casings and flavorants in tobacco is a function of the temperature of application. Work continues to define those optimum temperatures of application for casings and flavorants that would maximize their absorption into tobacco.

TOBACCO ADHESIVE STUDIES (T. E. Majewski)

A sucrose/calcium acetate composition has been identified that will reduce loose ends in the foamed rod injection process. Work is under way to modify the sucrose/calcium acetate composition chemically so that similar improvements in the cigarette rod firmness can be realized.

POLYPROPYLENE FILTERS ADHESIVES STUDIES (T. E. Majewski, A. Robinson, V. P. Henderson)

Work continues in characterizing the adhesives used for the production of FML polypropylene filter tow. Investigations are presently under way to validate the critical surface tension (γ_c) value previously obtained with a homologous series of methanol/water solutions in comparison to the literature value of methylene di-iodide/water solutions. The appropriate (γ_c) validation should allow us to make adhesive formulation recommendation changes to respective adhesive vendors.

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A. Robinson

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CHARGE NUMBER: 1708

PROJECT TITLE: Physical and Chemical Properties of Tobacco

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: H. A. Hartung

DATE OF REPORT December 6, 1985

I. Mechanical Properties of Treated Papers

Objective: To characterize papers treated with foam binding agents.

Status: At heavy loadings of foam binding agents there was no appreciable effect on the stiffness of papers. This showed that the binders operate by gluing shred contacts and not by increasing shred stiffness. No further work is planned.

II. Impact Breakage Studies

Objective: To explore the effects of additives on the impact breakage of tobacco shreds.

Status: Data have been obtained on RLTC and RL 150-B samples plasticized with PG, PG/glycerine or TEG (as controls). RL 150-B showed much less degradation at equilibrium moisture because it had considerably higher OV levels. In the RLTC system, PG/glycerine was about the same as the TEG controls while PG alone was clearly inferior.

Plans: Compare laboratory breakage data with large scale survivability studies.

III. New Plasticizers and Humectants

Objective: To unearth new materials for screening as plasticizers and humectants for smoking products.

Status: Preliminary literature reviews have identified 60 candidates for further study.

Plans: Complete search and review steps. Select most promising candidates for further studies.

H A Hartung

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PROJECT NUMBER: 1720

PROJECT TITLE: Physiochemical Morphology

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: E. Thomas

WRITTEN BY: H. Nakatani

DATE OF REPORT: December 6, 1985

Objective: To determine the biochemical and biophysical properties of chloroplast submembrane preparations with respect to oxygen evolution and elucidate the degradation pathways of chloroplast proteins as a function of senesence. (V. Baliga and H. Nakatani)

Status:

Chloroplasts of a clover mutant could not be detergent fractionated in terms of PSII and PSI. This suggests a different structural localization of the photosystems in the membrane the mutant versus that in wild-type material. V. Baliga presented the results of the clover studies at Philip Morris R&D and at the First Intl. Congress of Plant Molecular Biology (Savannah, Georgia, Oct.27-Nov.2,1985). Radiolabelling (125I and 3H) studies on PS II submembrane preparations have been approved at MCV (Dr. Larry Shook) through the coordinated efforts of A. Frisch, R. Izac, B. Edwards and B. Davies. MCV facilities will also be used to conduct control experiments on non radioactive preparations. Results of studies on tobacco chloroplast senescence show a decrease in the LHCII complex (SDS-PAGE profile) which was correlated with an increase in the chlorophyll a/b ratio. Decrease of the alpha and beta subunits of the coupling factor were found and correlated with the inability of the chloroplast to form a pH gradient. Corrected absorption spectra for chlorophylls a and b (Sigma) were obtained and the wavelength drives of the Farrand Fluorimeter were recalibrated.

Plans: Dr. Andrew Staehelin (University of Colorado, Boulder) has agreed to collaborate in studies to examine the topological architecture of PS II and PS I by immunocytochemical techniques. Antibodies are to be provided by Dr. N.H. Chua (Rockefeller University, New York) and Dr. C.J. Arntzen (DuPont, Wilmington, Delaware). Trifluoromethyl(125I)iodophenyldiazurine will be tested initially to label Ca-sensitive proteins in PS II submembrane preparations associated with oxygen evolution and examined by autoradiography at MCV. Further studies are being continued to reduce the LHCII component in the PS II preparations.

The variability of damaged photosynthetic components in the senescence study suggests a requirement to monitor the structural integrity (electron microscopy) of the cellullar matrix as a function of senescence rather than to rely simply on leaf coloration and position.

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Evaluation of isolation and assay methods for carboxypeptidase and leucine aminopeptidase in Project 1904 have been made to try to optimize activity measurements.

Objective: Study the physical and chemical properties of green tobacco and relate them to the mechanical properties of cured leaf. (E. Taylor, E. Thomas, J. Lyle, P. Echlin)

Status: Sixty of the 102 leaves from the summer of '85 greenhouse growth study have been analyzed for elemental distribution using the ZAF/PB ratio method. All of the leaves from the Sr and Rb-spiked tobacco plants and control leaf materials have been examined by EDS. Digimapping was also performed on the control leaf material for Ca and K, and on Sr and Rb-spiked material. (Note: Sr and Rb are assumed to be biologically analogous to Ca and K, respectively.) The intensities of K and Rb were closely correlated and were localized in the vacuoles of the upper epidermal cells. The cell wall had relatively little of these latter two elements. Sr and Ca were distributed uniformly throughout the different tissue types. An LVDT (linear variable differential transformer) was obtained from S. Ganeriwala and calibrated to obtain strain measurements with the tensile stage apparatus. The Young's modulus for a rubber sample was determined and agreed with published values. Jackie Lyle attended a course on electron microscopy and X-ray microanalysis at the State University of New York, New Paltz, N.Y. (S. Edith Taylor is providing on-going training on STEM and TEM to J. Lyle at PM).

Plans: The data for the summer leaf material and the Sr and Rb-treated material are to be stored in the computer matrix for further analysis. Continue tensile stage studies on tobacco material with experiments to determine the Young's modulus on Bright, ripe tobacco leaves.

Service Work:

Soot samples from the parking lot area and neighboring stemmery have been examined by EDS for Bob Carpenter: a package of punctured Va Slim cigarettes was visually examined. No discoloration nor residue (by EDS) was found on the paper or tobacco. The hole sizes were determined to be 750 +/- 50 microns.

Polyclonal antibodies raised against nitrate reductase (NR) from squash have been obtained from Dr. W. Campbell (Michigan Technological University) to attempt to identify the presence of NR in various tobacco samples (Project 6902) by Western blotting techniques (this technique assumes that the antibody against the squash NR will react with the tobacco NR).

Beverly Nakatani

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CHARGE NUMBER: Project 1730

PROJECT TITLE: Plant Tissue Research

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: L. Weissbecker

DATE OF REPORT: December 6, 1985

I. Objective: Produce tobacco plants with significantly reduced leaf Cd levels (Sanders).

Status: A Coker 319 haploid cell line (Cd 4) was grown in suspension culture and several cell concentrations were plated on solidified media containing various concentrations of Cd. Dose response curves were obtained that varied depending upon cell and Cd concentration. The response was quantitated using Project 1720's image analyzer system to measure regenerated callus area. The curves differed depending upon the cell concentration plated. When 0.288 gm of cells were plated, the reduction to one half of the regenerated callus area occurred at about 45 ppm of Cd. When 0.144 gms of cell were plated the Cd concentration needed to reduce the regenerated calli area by 50% was about 16 ppm. No calli were regenerated when 0.072 gm of cells were plated even in the absence of Cd showing the dependence of calli regeneration on a certain minimum number of cells plated.

Plans: Cells will be plated at a fixed concentration to avoid any possible cell-Cd interaction.

II. References:

K. Sanders - notebook #8165.
M. Shulleeta - notebook #8228.

L Weissbecker

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CHARGE NUMBER: Project 1901

PROJECT TITLE: Biochemical Modification of Tobacco

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: D. M. Teng

DATE OF REPORT: December 6, 1985

I. Inhouse Air-Curing of Burley Tobaccos

Air-curing of burley tobacco under different moisture levels was completed. Samples are being stemmed, and will be submitted for analyses when they are stemmed. One of the samples had very distinctive ammoniacal and barnyardy characters. It will be analyzed to determine the source of barnyardiness.

II. Bright Tobacco Studies

Bright tobacco which were flue-cured under different conditions to facilitate some of the protein breakdown were stemmed, and submitted for analysis.

The filling values (C.V.) of freeze dried samples of mid-stalk tobacco from different stages of growth were determined. Samples taken two weeks before topping had the lowest C.V., and the samples taken at two-week intervals afterwards did not show much difference, except the sample at fully ripe stage which was the highest. This has to be repeated to confirm the finding and the causes of the trend. Samples were submitted for analysis.

Once-over harvested bright tobacco leaves (control and Ethrel-treated) which were either freeze dried, Semiwork dried or flue-cured were submitted for analysis. Ethrel-treated flue-cured sample had higher filling value than flue-cured control. This has to be repeated to confirm the result.

DM Teng

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CHARGE NUMBER: Project 1902

PROJECT TITLE: Tobacco Microbiology

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: D. K. Chadick

DATE OF REPORT: December 6, 1985

I. TEG Solvent Replacement in SEL:

Objective: Evaluate suitable solvents to replace TEG in SEL. The selected solvent should be compatible with the propylparaben preservative and act as a humectant in the finished sheet.

Status: Another flask-level experiment was performed using 1.5% TEG, 1.8% propylene glycol (PG), and 1.8% PG + 1.5% glycerin. The levels of propylparaben were as follows: 100, 200, 300, and 400 ppm. All solvent/humectant percentages were on a (v/v) basis. SEL without class tobacco was prepared in the C Pilot Plant. The SEL was sterilized and inoculated with a 10% (v/v) mixture of the known spoilage bacteria in a 1:1:1:1 ratio. The flasks were incubated four days at 37.5°C at 150 rpm. Three controls were also included in this study, one flask contained only sterile SEL, one contained sterile SEL with a 10% inoculum of the bacterial mixture, and the third was the series of flasks with the solvent/humectants along with a 10% inoculum of the bacterial mixture.

The results from this experiment showed no significant differences in bacteriastasis between the various solvent/humectant and preservative combinations that were tested. The reason(s) for this is not clear at this time. We have observed that the growth characteristics of the four known spoilage bacteria are quite variable in the SEL medium lacking class tobacco and no additional solvents/humectants or preservatives.

Plans: Conduct growth experiments to elucidate the behavior of the control cell cultures. Apply this information to future studies involving solvents/humectants and preservatives.

II. Humectant Replacement Trials in C Pilot Plant:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: Samples of the acceptable sheet are currently being stored in our environmental rooms at 37.5°C and 25°C with 80% RH maintained at both temperatures. The sheet is being analyzed for bacteria, and fungi (yeasts and mold) at 1, 2, 4, 8 and 12 week intervals. The first monthly data is as follows:

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TABLE 1

C Pilot Plant RL-TC

Time	Code	Bacterial Colonies/g($x10^6$)	Mold Colonies/g
0	1254[1]	19	80
	1255	28	165
	1256	24	80
	Control[2]	2.5	5
4 Weeks	1254	22	15
	1255	20	5
	1256	17	0
	Control	230	0

[1] 1254 - 3% PG + 2.5% Glycerin
1255 - 4% PG
1256 - 4.6% TEG
All test sheets were prepared with 700-800 ppm propylparaben.

[2] The control was Park 500 RL-TC with 4.6% TEG and 700-800 ppm propylparaben.

Note: No preliminary conclusions will be made at this time.

TABLE 2

C Pilot Plant RL-150B

Time	Code	Bacterial Colonies/g($x10^6$)	Mold Colonies/g
0	123B[1]	6.3	50
	1264A	5.1	80
	1265B	14	25
	1266C	20	15
	Control[2]	3.0	5
4 Weeks	1263B	18	5
	1264A	6.2	5
	1265B	1.0	5
	1266C	10	5
	Control	9.0	0

[1] 1263B - 3% PG + 2.5% Glycerin
1264A - 4% PG
1265B - 5% PG
1266C - 4.6% TEG
See Table 1 for propylparaben levels.

[2] The control was Park 500 RL-150B with 4.6% TEG and 700-800 ppm propylparaben.

Note: No preliminary conclusions will be made at this time.

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Plans: This is an ongoing study and the data will be reported monthly, based on the storage time of the various sheets.

III. Humectant Replacement Trials at Park 500:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: The sheet collection was completed on October 24th, and the samples are being treated as mentioned in item 2. In addition to the storage study done in our environmental rooms, hoghheads of sheet will be stored in a warehouse. At appropriated time, they will be opened and samples will be removed and analyzed as stated in item 2.

Plans: As previously mentioned in item 2.

IV. Humectant Replacement Trials at the RCB Plant

Objective: Store RCB that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: Sheet storage has begun as previously described in item 2.

Plans: As previously mentioned in item 2.

V. Miscellaneous

Objective: Assist members of Analytical Group with an RL sheet storage experiment (3).

Status: Samples of ground tobacco provided by B. Harvey and G. Baker were sterilized and aseptically packed into gelatin capsules. The capsules are currently being stored in one of our incubators at 37.5°C and periodically being sampled for changes in the acetic acid level.

Plans: Assistance in future studies as needed.

References

[1] Chadick, D.; Monthly Report, Project 1902, PM Monthly Summaries, Accession #85-221, 1985, November, 15.

[2] O'Neill, J.J.; Mead, C.A. The parabens: Bacterial adaptation and preservative capacity. J. Soc. Cosmet. Chem. 33: 75-84; 1982.

[3] Baker, P.G.; Harvey, W.R.; The determination of acetic and boric acids in RL sheets by ion chromatography. PM Special Report, Accession #IR85-217: 1985, November, 4.

D. L. Chadick

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CHARGE NUMBER: Project 1904

PROJECT TITLE: Tobacco Physiology and Biochemistry

PERIOD COVERED: November 1-30, 1985

PROJECT LEADER: I. L. Uydess

WRITTEN BY: K. Rapp Sherwood

DATE OF REPORT: December 2, 1985

Objective: To establish the time course and biochemical changes characteristic of tobacco leaves at various stages during senescence.

Status:

I. Enzyme Assays and Associated Methodologies.

In an attempt to demonstrate endopeptidase and/or carboxypeptidase activity in fresh, green, bright (Coker 319) leaf, extractions were prepared at four different pHs (pH 5, 6, 7, and 8). Each extract was also concentrated 10-15 X using an Amicon stirred ultrafiltration cell with a filter molecular weight cutoff of 10,000. When these extracts and their concentrates were tested, no activity was found for either carboxypeptidase A or B; and only the concentrated extracts appeared to have activity in the endopeptidase assay. Although it required a concentration step, this was the first time endopeptidase activity in tobacco extracts had been demonstrated in our lab. A hint of activity for the concentrated extracts was also found when these extracts were screened in an endoproteinase fibrin clot assay marketed by Boehringer Mannheim. As with all previous extracts, leucine aminopeptidase activity was present in all of the extracts tested.

Since we were unable to detect carboxypeptidase activity in these extracts, a study was conducted in collaboration with H. Y. Nakatani to look at protein degradation in one of the concentrated extracts. A portion of the pH 8.0 concentrated extract was incubated at 37°C for 24 hours after which a 3-27% gradient sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) was run using the control (extract stored frozen at -20°C) and the incubated sample. It was expected that proteolytic activity during incubation would be evident by the appearance of low molecular weight fragments in the incubated sample. Instead, it was found that a large amount of the protein in the incubated sample compared to the control was not getting into the gel. Condensation reactions between proteins or protein fragments with phenolics and other oxidation products could explain this observation.

Since endopeptidase activity in leaf extracts prepared from *Nicotiana rustica* had been reported by Weckenmann and Martin (1984), their published extraction and assay procedures (which were different from the procedures previously used in our laboratory) were reexamined as closely as possible using fresh, green leaf from *N. rustica* and Coker 319 tobaccos. Endopeptidase activity was subsequently demonstrated for both of these extracts. This activity was confirmed in several successive experiments. This was the

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first time that endopeptidase activity had been repeatedly demonstrated in a tobacco extract in this laboratory without going through a concentration step. When the pH 7.0 extract and its concentrate (see above) were again tested according to the Weckenmann and Martin assay procedure, only the concentrated extract showed endopeptidase activity as had been observed before. Activity was also found when an extract was prepared according to the Weckenmann and Martin procedure from a leaf that had been frozen and stored at -80°C from the summer of 1984. Additional experiments are currently underway to optimize the assay running conditions. Furthermore, testing the substrate (azocasein) in 50 mM phosphate buffer, pH 4.5, was found to be better than testing in pH 4.5, 50 mM citrate/phosphate buffer as suggested by Weckenmann and Martin. A 2 hour incubation time for the assay was also found to give substantially higher values than a 1 hour incubation for the tobacco extracts. A time course study is planned to find the optimum incubation time for the assay with tobacco extracts. The results from a pH optimization experiment for assay running conditions showed an optimum in the range of pH 4.5 - 5.5. This experiment will be rerun to confirm these results. Experiments to study the effects of freezing on activity are also underway. Plans also include a study of the effects of certain protease inhibitors, and potential extraction components (e.g., ascorbic acid, thiourea).

Differences between leucine aminopeptidase and endopeptidase activity from extracts prepared from fresh green and fresh senesced leaf (Coker 319) have been demonstrated. When the enzyme activities were calculated on a unit of activity/hour/mg of protein basis, the leucine aminopeptidase activity was shown to decrease in the senesced leaf confirming our earlier results, whereas, endopeptidase activity was shown to increase. The latter was supported by similar results reported by Weckenmann and Martin for N. rustica.

II. Immunochemical Phytohormone Assays (In collaboration with B. Davies).

Aqueous, 80 % methanol extracts of greenhouse grown green and senesced leaf (Coker 319) were prepared for testing and methods development of an enzyme-linked, immuno-sorbant assay for abscisic acid and indole acetic acid. Transfer of these procedures to Project 1904 will occur in December, 1985.

Karen Rapp Sherwood

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PROJECT CHARGE: 2106

PROJECT TITLE: APPLIED TECHNOLOGY

PROJECT LEADER: J. L. Banyasz

PERIOD COVERED: November 1 - 30, 1985

DATE OF REPORT: November 25, 1985

PACKAGING (J. L. Banyasz, C. D. Owens and T. V. Van Auken)

In collaboration with Development, samples of paper with microencapsulated water were tested for water release as a function of ambient RH. Only small weight changes were observed which were consistent with changes in the moisture content of the paper itself. The capsules apparently lost their moisture content during the application process.

Work is proceeding on the relative humidity indicator to be used inside cigarette packs as an indicator of pack OV. Discussions with Thide-Engdahl concluded that it is highly probable that a rotogravure ink can be formulated to include such an indicator system.

Based on the barrier properties of overwraps summerized in previous reports and the sorption isotherms of tobacco a mathematical model was developed to predict the rates of moisture loss or gain from cigarette packs as a function of ambient RH. An overview of this work was presented at a recent Focus Goal #2 review. Experiments to test the validity of the model are under way.

FLAVOR TRANSFER (A. L. Finley)

A set of conditions for the reproducible and reversible activation (dehydration) of 150 A silica gel have been developed. This consists of the application of at least 25 in Hg of vacuum for at least eight hours followed by the slow application of heat at a rate of 5-10°C/minute up to 175°C. Exceeding 225°C appears to cause irreversible damage to the gel. The duration of heating depends on sample size. Flavor addition to the 150 A gel is proceeding. However, data to this point indicate that large gel samples will require heating times that may be too long to be practical given the present procedure.

Plans have been made to investigate the flavor release mechanism of the PVA flavor storage/release system in collaboration with Development.

/ev

J. L. Banyasz

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CHARGE NUMBER: 2501

PROJECT TITLE: Smoke Chemistry

PROJECT LEADER: R. H. Newman

PERIOD COVERED: November 1-30, 1985

DATE OF REPORT: December 4, 1985

NON-COMBUSTABLE SMOKING ARTICLE

Preliminary gc experiments indicate that a mixture of thiazoles, an oxazole and a tobacco extract all diminish in delivery 10 fold in 10 puffs. As would be expected the more volatile components are reduced faster relative to the less volatile ones. The delivery vehicle was quartz wood. Due to the large RTD, fine mesh bonded phase silica gel was not satisfactory.

PROMPT GAMMA ANALYSIS

Samples of 2R1 tobacco filler and smoke condensate were analyzed by prompt gamma-ray analysis. The elements H, B, C, N and P which cannot be analyzed by neutron activation analysis can be detected by prompt gamma. The following data was obtained for percentage delivery to condensate calculated from the expression.

$$\% \text{ delivery} = 100 \frac{\text{condensate} \times 0.03}{\text{tobacco} \times 0.73}$$

Where 0.03 is the average condensate delivery to mainstream smoke and 0.73 is the fraction of cigarette burned.

CONDENSATE DELIVERY

ELEMENT	TOBACCO	CONDENSATE	% DELIVERY
B	30 ppm	1.2 ppm	0.16
N	2.3%	0.6%	1.1
Si	2%	0.4%	0.4
S	0.6%	0.3%	2.0
Cl	0.7%	0.2%	1.2
K	3.6%	0.2%	0.2

MASS SPECTROMETRY

Pyrolysis/gc/ms was carried out on a menthol release compound at 300°C and 600°C. At both temperatures menthol and menthene were identified.[1]

Yucca extract was analyzed by pyrolysis/gc/ms. The volatile products at both temperatures were of very low yield considering the amount of material (1-2mg) pyrolyzed. The results were documented in a recent memo.[2]

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References:

1. Naworal, J.; Greene, F., Pyrolysis GC/MS Analysis of M-1005. Memo to Roger Comes, November 27, 1985.

2. Naworal, J.; Greene, F., Pyrolysis GC/MS of a Yucca Extract. Memo to Dr. J. L. Charles, December 2, 1985.

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CHARGE NUMBER: 2525

PROJECT TITLE: NATURAL PRODUCTS CHEMISTRY AND BOTANICAL INVESTIGATIONS

PERIOD COVERED: NOVEMBER 1 - 30, 1985

PROJECT LEADER: HARVEY J. GRUBBS

DATE OF REPORT: DECEMBER 10, 1985

I. ORGANIC CHEMISTRY

The development of new synthetic methods for the preparation of tobacco identical glycosides continues. Benzyl 4,6-di-O-acetyl-2-O-benzoyl-α-D-mannopyranoside was treated with α-D-mannose pentaacetate in methylene chloride and excess boron trifluoride etherate to give a 75% isolated yield of benzyl 4,6-di-O-acetyl-2-O-benzoyl-3-O(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside. This experiment demonstrates the utility of the boron-trifluoride etherate mediated glycosidation reaction in the synthesis of disaccharides. This method is an improvement over classical methods which require the tetra-O-acetylmannosyl bromide and heavy metal salts.

Additional quantities of phenethyl β-D-glucopyranoside and guiacyl β-D-glucopyranoside have been prepared for smoke studies and subjective testing.[1]

Synthesis of additional quantities of the cigarette beetle pheromone (± serricornin) continues.[2] Approximately 14g of pheromone was supplied to Project 1101 for additional field testing experiments.

Work continues on the synthesis and characterization of sclaral ether flavorants. This class of flavor compounds, generally described as "woody" character materials, shows much promise as tobacco flavorants. An invention record, with appropriate chemical and subjective characterizations, has been prepared for filing with the Patent Office.[3]

The preparation of a specifically labeled menthol glycoside has been completed.[4] The preparation involved reaction of (1,3-^{14}C-propyl)-menthol with acetobromoglucose, followed by deacetylation with sodium methoxide in methanol. The yield of (1,3-^{14}C-propyl)-menthol β-D-glucose was 3.7 μCi.

II. NATURAL PRODUCTS CHEMISTRY

Work continues on the preparation and characterization of radiolabeled tobacco identical hydrocarbons.[5] As part of a program to investigate the smoke chemistry of this class of compounds, separations methodology is currently under development. At ambient temperature adequate resolution and peak shape was obtained in the HPLC analysis for alkanes smaller than n-C_{32}. The effect of raising the column temperature on resolution and peak shape is being investigated. The column temperature tested was from 31° to 50°C using an Altex Ultrasphere ODS column on a Rainer LC column heater. Alkanes tested

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included n-C_{20} thru n-C_{40}. A small loss in resolution and a significant improvement in peak shape was observed as the temperature was increased. The improvement in the peak shape was sufficient to allow for the detection of 1-5 μg quantities of n-C_{30} and n-C_{40} using the FID detector.

Efforts continue in a cooperative tobacco breeding study with Dr. E. Wernsman at NCSU (North Carolina State University).[7] Green leaf disks (2.5 cm) from approximately the sixth leaf of each plant in burley families originating from crosses of (Galpao Coumn x Greenville 115) S1 x Kentucky 14, suspected to be segregating for abienol synthesis capability, were collected and analyzed for cis abienol. As the plants flowered they were topped, but not suckered. Upon completion of leaf disk abienol analyses[6] (approximately 3.5 weeks after topping) pollinations were made on specific plants of interest in each family, while remaining plants, both abienol producers and non-producers, were closely hand suckered. Families of plants were stalk cut, speared, and hung in a highly ventilated curing shed. This was 21 days after plants had been suckered and last sidedressed, and six weeks following topping.

Following the completion of curing, leaves from all abienol synthesizing plants were stripped, separated into two stalk positions (midstalk and tips), and leaves from multiple plants in each family composited. The leaves were tied and tagged to identify their family, abienol content, and stalk position, and stored in plastic bags. Leaf from plants in low abienol content were handled in a similar manner.

Observations of plants during the yellowing phase of curing revealed unique characteristics of these tobaccos compared to traditional burley. Complete chlorophyll degradation in traditional burley results in a pale yellow leaf. These tobaccos posess a rich orange color at the end of yellowing. In stripping the leaf, it was noted that the tobaccos were exceedingly sticky, and that the exudate accumulating on the worker's hands imparted a very strong, sweet, fruity, and pleasant aroma. Leaf midveins and plant stalks also possessed an unusually high quantity of what appears to be trichome exudate.

Leaf color varied with stalk position from buff tips, reddish-brown leaf, to red tips. Leaves possess very good order-holding capacity, an excellent finish, and are exceedingly elastic. The quantity of oil in the leaf is very high. Cursory glances of adaxial leaf surfaces suggest variegated areas of the lamina; in reality these were sections of oil extrusion due to compression. The leaf has a pleasant tobacco aroma, particularly the abienol producers, which is different from that of a typical burley tobacco.

Approximately 35 lbs. of cured leaf has been obtained from NCSU. These samples are currently being evaluated for leaf chemistry, physical properties, and subjective qualities.[8,9]

Harry Gribb

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III. REFERENCES

1. G. Chan

2. D. Williams

3. C. R. Howe

4. R. Izac

5. S. Hassam

6. M. Core, R. Izac

7. R. Bass, G. West, G. Newell, R. Izac, M. Core

8. Grubbs, H. J.; NCSU (North Carolina State University) Cooperative Research Program. Memo to Distribution, November 1, 1985.

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Research

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CHARGE NUMBER: 1101

PROGRAM TITLE: ENTOMOLOGICAL RESEARCH

PERIOD COVERED: NOVEMBER 1 - 30, 1985

PROJECT LEADER: D. L. FAUSTINI

DATE OF REPORT: DECEMBER 5, 1985

I. CIGARETTE BEETLE PHYSIOLOGICAL STUDIES

A. Field

The study to evaluate the efficacious effects of methoprene on cased Mexican tobacco has been completed. The results showed no adult emergence when larvae were fed on the methoprene treated tobacco. An average of 12 ppm methoprene (n = 28) was determined from residue analyses (1).

The study to evaluate the efficiency of CB monitoring in warehouses with standard light traps vs. Serrico® pheromone traps has been concluded. Large warehouses were selected since their size contributed to lower efficiency of the standard traps. The minimum number of pheromone traps required in various volume warehouses and the optimum trap location was also determined in the study. The results showed that the Serrico® traps were more efficient monitors than the standard traps. No significant difference in the number of CB trapped was observed, regardless of the number of traps employed in the warehouse. Therefore, a minimum of two Serrico® traps is being recommended for each warehouse. The location of the trap was found to be significant; that is, those located near the entrance doors trapped more CB (2).

Two efficacy tests were conducted on the AMF-Legg conditioning unit in OC Primary at the request of Engineering. The results showed 100% mortality of all life stages tested (3).

B. Laboratory

CB collected from "Kabat" tobacco warehouses and reared on methoprene treated tobacco did not produce subsequent generations. This suggests that Kabat® resistance was not a factor in the infestation experienced in the Kabat® warehouse during 1984-85, and that it was probably due to other factors (4).

II. ASSISTANCE TO OTHERS

One hundred percent CB mortality was achieved at the 20th St. conditioners used for phytosanitary certification of export blended tobacco (5).

A study was completed to evaluate the current status of pesticide management by our affiliates and to recommend improvements in existing programs and/or develop new formalized programs (e.g., proper storage and handling). The study was a joint effort by Leaf Dept., TTG, International and R&D (6).

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At the request of Louisville QA, a recommendation was made not to fumigate the Annex building with MeBr. Based upon CB trap records other alternatives were offered (7).

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2. Minor, M. F. Memo to Mr. J. Thomas, "Comparison of Pheromone Traps and Standard Black Light Traps in Monitoring Cigarette Beetle Populations in Warehouses." November 22, 1985.

3. Minor, M. F. and Drew, S. Memo to B. Woodhouse, "Verification of Beetle Kill in AMF-Legg Conditioning Unit." November 22, 1985.

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7. Faustini, D. L. Memo to R. H. Bowling, "Tarpaulin Fumigation of Louisville Annex". November 25, 1985.

Daryl L. Faustini

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CHARGE NUMBER: 1620

PROJECT TITLE: ELECTROPHYSIOLOGICAL STUDIES

PERIOD COVERED: NOVEMBER 1 - 30, 1985

PROJECT LEADER: F. P. GULLOTTA

DATE OF REPORT: DECEMBER 4, 1985

WRITTEN BY: C. S. HAYES C.S.H.

I. EVENT-RELATED POTENTIALS (ERPs)

A. Difference Testing Employing Cognitive ERPs

In order to determine the effects of varying stimulus discriminability and inter-stimulus-intervals (ISIs) on the cognitive ERP, experiments employing auditory stimuli have been initiated.[1] Three ISIs (1.1, 5.3 and 11.1 seconds) and two levels of discriminability (easy vs difficult) are being utilized.

To date, results from one subject suggest that with respect to stimulus discriminability our previous findings have been replicated. That is, at each of the ISIs tested, the cognitive component elicited to the easy discrimination is larger than the component elicited to the more difficult discrimination. Perhaps even more interesting, however, are the data with respect to the various ISIs. At short ISIs the traditional finding has been confirmed in that the cognitive component appears to only be elicited in response to target stimuli. However, at long ISIs a cognitive component is elicited to standard stimuli as well as target stimuli. Under these conditions it seems that the passage of time (i.e., absence of stimulation) serves as the background against which targets as well as standards are compared. In fact, under the long ISI condition, the experiment appears to take on the characteristics of a two-target experiment, with the absence of stimulation serving as the standard stimulus and the standard and target stimuli serving as high and low probability targets respectively. These findings are analogous to those obtained in flavor discrimination experiments which require the use of long ISIs. Therefore, due to the implications of these findings for the interpretation of data from flavor discrimination experiments, our plans are to test at least one additional subject utilizing the auditory cognitive ERP paradigm in order to further investigate this phenomenon.

II. THE EFFECTS OF CO_2 ON CIGARETTE SMOKE FLAVOR

To date, three additional subjects have completed discrimination training and are currently being retested.[2] Retesting of these subjects should be completed by the middle of the first quarter of 1986.

III. REFERENCES

1. Wannamaker, I. Notebook No. 8262, pp. 64-121.
2. Hayes, C. S. Notebook No. 8150, pp. 122-126, 131-137.

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PROJECT NUMBER: 1740
PROJECT TITLE: Flavor Components of Tobacco and Filler
PROJECT LEADER: C. S. Kroustalis
PERIOD COVERED: November, 1985

I. FLAVORS

A. Two essential oils and two proposed synthetic substitutes were profiled for Operations Services. The substitutes contained trace levels of undesirable components.

B. A Guatemalan flavor (85-012-GUA) contained 1.4% CF.

C. Three Players Lights menthol flavor concentrates were profiled. Only one showed mostly quantitative differences which were attributed to starting ingredients batch differences by Flavor Center personnel.

D. Two proposed substitute flavors showed minor quantitative differences. The supplier will prepare new batches of these flavors using ingredients from the same batch.

E. Brazilian and Chinese mint oils and menthols derived from these oils by both the Brazilian and Chinese processes were profiled to determine whether differences could be detected. The differences were noticeable and one could determine the source of menthol. Steam distillates from the filters of Salem, Newport, Kool and B&H cigarettes were compared. The minor menthol isomers were identified by GC/MS (D. Magin) and were quantitated by T. Crews.

II. OTHERS

A. Samples of autoclaved RL which was inoculated with bacteria isolated from SEL by D. Chaddick and stored at 80% R.H. and 37 degrees C for a week were analyzed daily. Results showed that bacterial fermentation was taking place. The results indicate that sugars were converted to 2,3- and 1,3-butanediols in addition to increases of acetic and propanoic acids. Another indication of fermentation was the conversion of 2,3-butanediol to acetoin near the end of the cycle. Further work will be necessary to determine whether the subjective off-taste of spoiled RL is related to bacterial biomass increase.

B. Warehouse RL and replacement humectant study samples were screened for volatile fatty acids. Approximately 500 samples were analyzed during this reporting period.

C. Players Lights menthol filler and cigarettes were analyzed for residual ethanol. Ethanol concentrations were higher than nonmenthol fillers and cigarettes.

D. Players Lights menthol, Merit 100's and B&H 100's with fruity pack aromas contained higher than usual levels of printing ink solvents (propyl and isopropyl

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acetates, toluene and mesityl oxide). Also present were 2,4-dimethyl-1,3-dioxolane-2-methanol, 2,2-dimethyl-1,3-dioxolane-4-methanolacetate, acetonyl alcohol and acetoin. The dioxolanes derive from glycols and aldehydes or ketones while acetoin and acetonyl alcohol are the respective bacterial fermentation products of 2,3-butanediol and PG according to the Merck Index.

E. Two customer complaint samples were analyzed during this period. One contained no detectable contaminants other than physical damage to the pack. The other was contaminated with compounds found in cherry and strawberry flavor formulations (identified by D. Magin using GC/MS).

C. S. Kronstalis

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PROJECT NUMBER: 1752
PROJECT TITLE: Optical Spectroscopy of Tobacco and Smoke
PROJECT LEADER: R. A. Fenner
PERIOD COVERED: November, 1985

I. Measurement of Smoke Components

A. Formaldehyde in Mainstream Smoke

A sampling system was developed for the analysis of formaldehyde in mainstream cigarette smoke by tuneable diode laser spectroscopy. Development of a quantitative puff-by-puff procedure for the system is currently in progress. Preliminary results for Monitor #23 cigarettes show a total cigarette delivery on the order of 12μg with 50% to 60% of the delivery resulting from the first puff.

B. Ammonia in Mainstream Smoke

The gaseous and total ammonia deliveries in mainstream smoke were determined for the following menthol brands: Kool 85, Salem 85, Newport 85, and Benson & Hedges 100. The results from these analyses are given in the following table.

Brand	% Soluble Ammonia	Mainstream Smoke: Gaseous[1] Ammonia, μg/cigt.	Mainstream Smoke: Total[2] Ammonia, μg/cigt.
Kool 85	0.13	0.9±0.2	7.1±1.2
Salem 85	0.18	1.4±0.3	8.7±1.2
Newport 85	0.14	2.1±0.3	17.6±2.4
Benson & Hedges 100 Menthol	0.29	2.6±0.2	18.6±1.8

[1]Tuneable diode laser method
[2]Trapping of whole smoke in aqueous medium with subsequent analysis by ion chromatography

The data show that Benson & Hedges 100's and Newport 85's deliver approximately twice as much gaseous and total ammonia as the Kool 85's and Salem 85's. It is also of interest to note that Newport 85's deliver more ammonia in mainstream smoke than would be expected based upon the percent soluble ammonia in the filler.

II. FT-IR Evolved Gas Analysis

The tobacco blends of the four menthol brands noted above were examined using the evolved gas analysis technique. The results suggested that the Salem blend may contain a higher percentage of burley or a different grade of burley relative to the Kool, Newport and Benson & Hedges blends.

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III. Memos

A. "Infrared Analysis of Sample #7619-98," R. A. Fenner to Dr. A. Kallianos, November 20, 1985.

B. "Data on Four Menthol Brands from the C.I. Division," M. E. Parrish to R. H. Cox, November 21, 1985.

C. "FT-IR Evolved Gas Analysis of Menthol Brands," R. A. Fenner to Dr. R. Cox, November 22, 1985.

D. "EGA Analysis of Menthol Crystals," J. L. Hart to Dr. R. Cox, November 26, 1985.

R. A. Fenner

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PROJECT NUMBER: 1754
PROJECT TITLE: Spectroscopic Studies of Tobacco and Smoke Components
PROJECT LEADER: W. N. Einolf
PERIOD COVERED: November, 1985

MS (D. Magin, N. Einolf)

LTF-IIA cigarettes were made with each of the following components at the 10% level (as the β-D-glucopyranoside): phenol, hydroquinone, menthol and 6,7-dihydroxycoumarin. Each cigarette type was smoked, the TPM extracted with dimethylformamide (DMF), and an aliquot of the DMF solution was derivatized with BSTFA/1% TMCS. The TMS derivatives of the aglycone and intact β-D-glucoside were quantitated by GC using 4-t-butylphenol as an internal standard. The delivery of aglycone and glucoside in TPM are as shown, and are given as percent of available glucoside in the filler.

	% as aglycone	% as glucoside	% Total Transfer
phenol	1.76	8.90	10.66
hydroquinone	6.79	2.70	9.49
menthol	0.72	10.5	11.2
6,7-dihydroxycoumarin	3.11	0.14	3.25

These cigarettes were unfiltered. In separate experiments, filters were attached to the LTF-IIA cigarettes containing menthylglucoside, and the resulting TPM gave a 0.27% menthol and a 7.5% glucoside delivery.[1]

GC/MS samples were analyzed at the request of D. Williams, J. Paine, D. Howe, T. Crews and R. Carpenter.[2]

The Wiley Data Base transfer is complete to our SS 200 data system. We are currently rebuilding the Wiley library, however, since it required more disk space than was originally allotted.[2]

NMR (J. Wooten, R. Bassfield, J. Campbell)

^{13}C CP/MAS spectra were obtained on six burley tobacco samples obtained from Walter Hempfling. Each sample has been extracted by various methods to remove protein. Difference spectra were obtained with a spectrum of normal burley tobacco revealing the types of material removed from each sample. More significantly, the CP/MAS spectra showed the extent of protein remaining. Signals in the high field aliphatic region indicate that proteins rich in amino acids with neutral, non-polar side chains are least affected by all the extraction methods. One detergent extracted sample exhibited strong signals from the detergent in its MAS spectrum obtained without cross polarization. Although strongly retained by the tobacco, the detergent remains very mobile and displays narrow signals in the tobacco which are very similar to the signals observed in its D_2O solution ^{13}C spectrum.[3]

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^{13}C NMR of solid materials obtained with magic angle spinning but without cross polarization can selectively detect mobile components dispersed within the solid, such as solanesol in tobacco. An attempt was made to observe menthol on cellulose acetate by this method. Significant signals were observed from the cellulose acetate in addition to a very broad background signal centered about the aromatic region. The ^{13}C MAS spectra of cellulose (cotton linters) also exhibited signals from the anhydroglucose units of the polymer but no background was observed. The MAS spectrum of cellulose differs from the CP/MAS spectrum in that the narrow line components attributed to crystalline cellulose are absent as well as both the narrow and broad lines for C-4. Several different cellulose samples were examined including the insoluble residue from tobacco fractionation, raw cotton, cotton linters, Sigmacell, Avicel, and acetobacter xylinum pellicle. The most amorphous of these samples, the tobacco cellulose, exhibited the strongest signals while the most crystalline, acetobacter, exhibited no signals at all. The method appears to be selective for either the amorphous regions, end group anhydroglucose units, or both. A ^{13}C T_1 measurement showed that the C-6 (hydroxmethyl) signal decays significantly more rapidly than the remaining signals, reflecting a surprising degree of motional freedom. The proton MAS NMR spectra of the cellulose samples was also observed but only a very broad signal from the rigid lattice protons was detected. A pulse sequence was encoded that employs a Hahn echo to suppress the rigid lattice signals. Each of the cellulose samples that have been observed with the spin echo pulse sequence have shown a relatively narrow proton signal which apparently corresponds to the same cellulose component detected in the ^{13}C MAS spectra.[3]

New data were obtained on the kinetics of the cooked flavor reaction which was monitored by ^{13}C solution NMR. By adjusting the solution concentrations and carefully adjusting the spectrometer, spectra were obtained with better signal to noise and stability than previously obtained. The relative concentrations of glucose, fructose, glucosyl amine, diglucosyl amine, and deoxyfructosazine were followed over a two day period. The data are being analyzed by John Lephardt.[3]

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1. W. N. Einolf, Notebook 8264.
2. D. F. Magin, Notebook 8231.
3. J. Wooten

W. Einolf

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PROJECT NUMBER: 1756
PROJECT TITLE : Analytical Sensory Correlations
PROJECT LEADER: B. W. Good
PERIOD COVERED: November 1985

I. RL vs. RCB SMOKE

The organic gas phase (FID and NPD) and neutral, basic and acidic fractions of the ten RL/RCB samples were analyzed by descriptive analysis (BMDP7D) and factor analysis (BMDP4M). While there was large variability in each sample type, some differentiation was observed. All four production models of RL-like-RCB were analytically similar to each other. RCB and one of the plain RL samples appeared clearly distinguishable from the other 8 samples in one or more of the profiles. The identification of responsible peaks for the observed differentiation is in progress.

II. MENTHOL CIGARETTES

Four menthol brands cigarettes, Kool 85, Salem 85, Newport 85 and B&H 100, were compared analytically. The latter was additionally sampled with its dilution holes taped making a total of five sample types. Smoke data was collected for characterization and comparison by multivariate analysis. TPM components were chromatographically analyzed by steam distillation-fractionation and gas phase components by FID and NPD. Each sample was analyzed three times. The data, which is organized for BMDP analysis, contains intensity measurements of 226 TPM components and 125 gas phase components. The data analysis is in progress.

III. ODOR PROFILING

Two vanilla bean samples, Tahitian and Mexican, were received from the odor profiling panel (M. Jeltema and E. Southwick) for chromatographic comparison and identification. The samples were ground and steam distilled in duplicate. These two beans show significant differences in the contents of octenal, 1-octen-3-ol, hexanoic acid, guaicoal, anisaldehyde, octanoic acid, t-methyl cinnamate, methyl anisate, anisyl acetate, eugenol and anisyl alcohol. Tahitian beans contained about twice the amount of vanillin as the Mexican beans.

IV. LABSAM

A final report was written.

V. REPORTS

"LABSAM: A Standard Customizable Laboratory Management System at Philip Morris", Bennie W. Good, Acc. No. 85-226, November 15, 1985.

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PROJECT NUMBER: 1758
PROJECT TITLE: Tobacco Cell Wall Research
PROJECT LEADER: Gordon H. Bokelman
PERIOD COVERED: November, 1985

I. TOBACCO ADHESIVES (Howard Sun, Bill Ryan, Gordon Bokelman, Sue Wrenn and Ray Dawson)

A literature search conducted by Sue Wrenn indicates that commercial licorice may contain substantial quantities of starch, simple sugars, gums, and even cellulose and lignin, in addition to glycyrrhizin. It appears that the composition of licorice is variable and its components are not well characterized. In order to better understand how tobacco binders based on licorice and pectin function, we intend to characterize commercial samples of block and spray-dried licorice by a modified version of our standard fractionation procedure (Bokelman, Gordon H., William S. Ryan, Jr. and Elisabeth T. Oakley, "Fractionation of Bright Tobacco," J. Agric. Food Chem. 1983, 31, 897-901).

Large scale chromatography of the di-sodium EDTA extract of flue-cured E-55 lamina using a QAE-Sephadex A-50 column yielded several grams of tobacco pectin. A portion of this sample was saponified to produce a de-esterified pectin. Both of these samples were submitted to Dr. George C. Ruben at Dartmouth College for the study of tobacco pectin gels using transmission electron microscopy.

Ray Dawson has suggested that we consider the use of "non-fractionated vegetable materials as combined adhesive, stiffener and humectant" for low-density rod cigarettes. His suggestion is based in part on work performed at Lancaster Laboratories on the stabilization of alfalfa hay pellets formed by compression. This stabilization involved producing a binder by enzymatic degradation of endogenous starch (see U.S. Patent No. 3,420,671). As a partial implementation of Dawson's suggestion, we have solicited samples of barley, malt and spent malt from Bill Herwig at Miller Brewing so that we may examine their starch contents.

II. CIGARETTE LOOSE ENDS (Bill Ryan)

Cigarettes were obtained from Peter Martin that had been produced by the Molins Company in such a manner as to reduce loose ends. Light microscopy suggested that the ends of these cigarettes may have been treated with a viscous solution that dried to form an amorphous coating. After storage under conditions of high humidity, it was soon apparent that the strands of tobacco at the end of the rod were no longer bound to one another. The first 5mm of tobacco obtained from the ends of several of these cigarettes are now being analyzed for sugar content.

III. RECONSTITUTED TOBACCO STUDIES (Bill Ryan)

More progress has been made in a study to examine the chemical compositions of commercial samples of RCB, RL and RL baseweb. The following additional results (reported on a percentage dry weight basis) have been obtained.

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Component	RCB	RLTC	RL-BW
Pectin	13.2	15.2	27.7
Starch	0.82	<0.45	0.61
Lactic Acid	<0.7	1.1	<0.7
Oxalic Acid	1.8	1.4	2.3
Malic Acid	3.5	4.8	<0.7
Citric Acid	1.4	1.0	<0.7
Total Reducing Sugars	<2.4	3.9	<2.3
Total Alkaloids	0.76	0.78	<0.4
Nitrate Nitrogen	0.32	0.42	<0.05
Soluble Ammonia	2.46	0.13	<0.12
Potassium	3.00	5.23	0.88
Sodium	0.05	0.07	<0.029
Calcium	2.49	2.50	2.71
Magnesium	0.53	0.58	0.29
Phosphorus	1.92	<0.11	<0.12
Silicon	1.88	<0.29	0.32
Chloride	0.49	1.21	<0.12
Sulfate	1.06	1.14	0.35
Total Ash	21.2	18.4	10.0

The relatively high value for lactic acid in the RLTC suggests that at least some fermentation has occurred. The presence of diammonium phosphate in the RCB is indicated by the high values for soluble ammonia and phosphorus. Likewise, the high silicon value is indicative of the relatively high sand content of RCB. The RLTC has much higher values for potassium and chloride than does RCB, which is indicative of the stem content of the starting material for RLTC. Interestingly, all three materials have comparable calcium levels although they vary significantly in their pectin contents.

IV. MISCELLANEOUS

1. Memo to Dr. Richard H. Cox from Gordon H. Bokelman, "Compositional Determination of Binary Lamina-Stem Mixtures," dated October 31, 1985.
2. Memo to Dr. Richard H. Cox from Gordon H. Bokelman, "Priority Research Projects," dated November 1, 1985.
3. Memo to Dr. Richard H. Cox from Gordon H. Bokelman, "Acquisition of an HP 5970 B Mass Selective Detector," dated November 6, 1985.
4. Ruben, George C. and Gordon H. Bokelman, "New Evidence Suggests that the Cellulose Elementary Fibril is Triple Stranded and Wound Helicoidally," poster presentation at the Annual Meeting, American Society for Cell Biology, Boston, Mass., November 10-14, 1985.

Gordon H. Bokelman

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PROJECT NUMBER: 6505
PROJECT TITLE: Special Investigations and Methods Development
PROJECT LEADER: W. R. Harvey
PERIOD COVERED: November 1985

I. Metal Determinations

Cadmium determinations continue on samples of grafted tobacco and also normal leaf samples in support of the cadmium uptake study. A microwave digestion oven from the SEM company is being evaluated for our digestion needs. Up to 12 samples can be digested at one time using nitric acid and hydrogen peroxide. Preliminary results are encouraging.

II. Cooked Flavor Support

A. Precook by Continuous Flow

Both glass and stainless steel coils plugged with the precook when left standing for 4 days at 95°C. No restrictions were in either of the coils.

B. HPLC Examination of Cooked Flavors

Best separation of cooked flavor constituents was achieved using an HP diol 200mm x 4.6mm column with a water-methanol gradient. More than 20 peaks were detected in the 200-60nm range. These peaks showed similar spectra with a primary absorption maxima at 272-274nm. Extraction with methylene chloride had no effect on the magnitude of these peaks. This indicates a large group of polar "azines" e.g. pyrazines. The two largest peaks were identified as 2,5 and 2,6 deoxyfructosazine. No additional information was obtained when the cooked flavor effluent from the Dionex ion chromatograph was examined by the HPLC detector.

C. IC Scans for Cooked Flavors

IC scans were done on six samples of cooked flavors. These scans are now being done by General Analytical.

III. Volatile Acid Determination in RL Sheet

In the development of an analytical procedure for acetic and formic acid in RL sheet, an apparent buildup of volatile acids was observed when RL was packed in gelatin capsules over a period of time (4 days). An attempt to repeat this with the same samples was not successful. The reasons for this are under investigation since autoclaving the samples would have indicated whether or not the volatile acid buildup was of microbiological origin.

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IV. Menthol Project

A total of eight cigarette filler samples were examined by ion chromatography. Anions and monovalent and divalent cations were determined.

V. X-Ray Flourescence Analysis (XRF)

Samples of lower stalk position tobacco leaves were ground in the ball mill and the sand isolated by nitric acid digestion. Particle size will be determined by Project 1720. Variation in particle size is a drawback to determination of sand (as Si) in tobacco leaf by XRF and it is hoped that the ball mill will reduce the sample to a more homogeneous state. SiO_2 added to bright monitor tobacco as standards in the 0.5 to 10% range shows scatter, probably due to lack of homogeneity. The pure element reference files for Si and interfering elements have been established. The quantitative standards file has been set up.

VI. Reports

1. Palmer G. Baker and William R. Harvey, Special Report, "The Determination of Acetic and Formic Acids in RL Sheet by Ion Chromatography," Accession Number 85-217, November, 1985.

W. R. Harvey

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CHARGE NUMBER: 6902

PROGRAM TITLE: BIOCHEMICAL SPECIAL INVESTIGATIONS

PROJECT LEADER: A. H. WARFIELD

PERIOD COVERED: NOVEMBER 1-31, 1985

DATE OF REPORT: DECEMBER 5, 1985

I. CURING STUDIES

A. BURLEY TOBACCO (1): The burley tobacco is now fully cured. The curing time was extended to 9 weeks to allow full development of TSNA. Most of the analytical data has been obtained, but not processed. The final results will be available shortly. Constant values were obtained after the first week of curing when the protein content was monitored on a dry weight basis during curing, indicating that proteolytic activity was essentially complete after the first week. Cell counts obtained by culturing leaf organisms on nutrient and tobacco-containing agar showed essentially no increase in the number of cells growing as a function of curing time. Nutrient agar was 10 times better at supporting growth than tobacco agar.

B. V-446 TOBACCO (1): The V-446 tobacco has also been cured in 9 weeks. Analytical data is not yet available for the cured lamina. These data will be available for processing shortly as well.

II. ORGANIC NITROSATING AGENTS (2)

When nitrosated tobacco wax fractions were allowed to react with 2-ethylpiperidine for 10 days at 37.5°C in ethyl acetate solution and then examined for the presence of N-nitroso-2-ethylpiperidine, positive results were obtained. Although the various combinations of wax and treatments have not all been looked at as yet, there is definite indication that tobacco wax constituents can form nitrosating agents (NSA). This suggests that agents such as this may be formed from nitrite and terpenes present in cells of the growing leaves. The nitrosation would then be postponed until the integrity of membranes is lost. Formation of nitrosamines could then occur during curing, even though nitrite is not present at detectable levels. This fits the data that has been obtained in the 1984 curing studies, and early indications are that the same pattern will be seen in the current studies.

Plans are now being made to further examine the nitrosating agent formation hypothesis. Experiments have been designed to investigate the effect of nitrate regimen on organic NSA, and TSNA formation. Other experiments may be designed to determine which types of compounds actually form the NSA.

III. SERVICE TO OTHER PROJECTS

A series of bright tobacco samples were submitted by Dan Teng for nitrate, minor alkaloids, and TSNA analysis (3). These samples had been subjected to various treatments, such as freeze-drying, other drying methods,

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flue-curing, and DET expansion. Also, a similar series that had been treated with Ethrel before priming was submitted. The results of these analyses were reported in a memo (4).

Nicotine and minor alkaloids were determined on a sample of Coker 319 tobacco that had been grafted onto tomato roots, field-grown to maturity, and flue-cured (5). Samples of low alkaloid bright and burley filler were also analyzed for nicotine and minor alkaloids as well as TSNA (6).

IV. REFERENCES

1. Warfield, A. H. Notebook 8196; Petri, D. Notebook 8006; Hansen, K. Notebook 8215; Yu, T. Notebook 8149.

2. Warfield, A. H. Notebook 8196.

3. Memo from D. M. Teng to C. Ellis and A. Warfield. Tobacco Samples from 1985 Studies. November 15, 1985.

4. Memo from A. H. Warfield to Dr. E. B. Sanders. Analytical Data on Coker-319 Samples from Dr. D. Teng. November 27, 1985.

5. Memo from A. H. Warfield and K. Hansen to Dr. E. B. Sanders. Nicotine and Minor Alkaloids on Tobacco Sample from L. Weissbecker. November 1, 1985.

6. Memo from A. H. Warfield and K. Hansen to Dr. E. B. Sanders. Analysis of Low Alkaloid Tobaccos for Alkaloids and TSNA. November 13, 1985.

A. H. Warfield

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CHARGE NUMBER: 6904

PROJECT TITLE: BIOLOGICAL METHODS DEVELOPMENT AND UTILIZATION

PERIOD COVERED: NOVEMBER 1 - 30, 1985

PROJECT LEADER: MIKE PENN

DATE OF REPORT: DECEMBER 4, 1985

I. V79 INHIBITION OF METABOLIC COOPERATION (IMC) ASSAY

The IMC testing of CSC obtained from the model cigarette sample X6D5BBL (BBL) was completed this month (1). All experiments showed a dose-related increase in activity in the range of 5-10 ug/ml. Statistical analysis indicated that the activity of BBL CSC was slightly higher than that obtained with the Kentucky Reference (2R1) CSC.

A series of experiments designed to define the activity of hydroquinone (HQ), a metabolite of phenol, were completed (2). Because of the inconsistent response of HQ in the IMC assay additional experiments were needed to obtain a definitive answer. Statistical evaluation indicated that HQ was moderately active in the IMC assay (slope = 16.3 % recovery/ug of agent/ml of treatment medium/3 day treatment).

To study the multistage effect(s) of promoters in the IMC assay, the activity of mezerein, a stage II promoter (3), was investigated. Although no toxicity was observed in the dose range (0.01-5ug/ml) tested, mezerein appears to be highly active. These preliminary results indicated that mezerein was as effective in inhibiting metabolic cooperation as the positive control, TPA. Experiments are being conducted to confirm these results, and the data will be reported next month.

A study was initiated to determine if CSC would inhibit the activity of a strong promoter (TPA) (1). Several doses of CSC, obtained from the Kentucky Reference cigarette (2R1), was tested simultaneously with one dose of TPA. In the absence of TPA, 2R1 CSC showed a dose-related increase in activity similar to that obtained previously. When both compounds were tested a significant increase in activity was observed in a dose-dependent manner when compared to TPA or 2R1 CSC tested alone. Experiments are now in progress to confirm and expand these findings.

To facilitate flow cytometric analysis of IMC results an experiment was conducted to evaluate the cytotoxicity of carboxyfluorescein diacetate (CFDA) (4). The results indicated that CFDA was not toxic throughout the dose range (0.1-100 ug/ml) tested. Experiments are now underway to test the effect of CFDA on metabolic cooperation.

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II. CELLULASE TREATMENT OF TOBACCO

Two anaytical procedures have been implemented and standardized to facilitate examination of cellulase activity (4). They include the hexokinase mediated assay for glucose concentrations and dinitrosalicylic acid assay for free reducing sugars. In addition, an assay to quantitate the endoglucanase activity in cellulase is currently being evaluated.

III. REFERENCES

1. Tickle, M. H. Notebook 8200, pp. 174-175.

2. Horn, J. L. Notebook 8229 pp. 122-125.

3. Slaga, T. J.; Fischer, S. M.; Week, C. E.; Klein-Szanto, A. J. Cellular and biochemical mechanisms of mouse skin promoters. Review Biochemical Toxicology. 3:231-281; 1980.

4. Davies, B. D. Notebook No. 8005, pp. 190-193.

Mike Lem

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CHARGE NUMBER: 6906

PROGRAM TITLE: BIOLOGICAL EFFECTS OF SMOKE

PERIOD COVERED: NOVEMBER 1 - 30, 1985

PROJECT LEADER: D. J. AYERS

DATE OF REPORT: DECEMBER 4, 1985

WRITTEN BY: L. H. THOMPSON

I. GLUTATHIONE DEPLETION ASSAY (GDA): EFFECTS OF CSCs FROM MODEL CIGARETTES ON THE GLUTATHIONE (GSH) LEVELS OF V79 CELLS (1)

Three experiments are in progress to evaluate the effects of freshly prepared CSCs (tested within one hour after preparation) from seven model cigarettes on GSH levels of V79 cells. Preliminary results from one experiment indicated that of the seven models, only one (LTF-5E) was distinctly different in that LTF-5E depleted more GSH than any of the other cigarette models. Confirmation of these observations will be made upon completion of this study.

II. GDA: THE EFFECTS OF ACROLEIN ON GSH AND OXIDIZED GLUTATHIONE (GSSG) LEVELS OF V79 CELLS (1)

Acrolein was evaluated in the GDA in a time course and dose response experiment to determine if its response would mimic that observed with CSC in the GDA, i.e., dose dependent and time independent. Analysis of variance (AOVO) from the overall data indicated that acrolein does mimic the kinetics shown for GSH depletion in V79 cells with CSC. As with CSC, there was no significant increase in GSSG levels with acrolein.

III. GDA: DETERMINING THE VARIATION IN THE HPLC (1)

An experiment was carried out to determine the level of variation in the HPLC analysis of GDA samples. Five HPLC determinations were made for each sample. It appears that the HPLC in our laboratory can measure the GSH levels with little variation within the samples.

IV. SALMONELLA/MICROSOME (S/M) MUTATION ASSAY: EVALUATION OF LYOPHILIZED S9 (2)

It was previously reported that lyophilized S9 obtained from Orgenics, Ltd. did not activate 2R1 CSC, but did activate 2AA at a level three times that observed with our standard AR20 S9 (3). To verify that lyophilization did not affect the enzymes responsible for the activation of CSC, but rather that the lack of response may be due to the source of the S9, a repeat experiment was performed with lyophilized S9 from Orgenics, Ltd. and from Litron. The results were as follows: 1) there was no activation of 2R1 CSC or 2AA using the lyophilized S9 from Orgenics, Ltd.; and 2) the activation of 2AA and 2R1 CSC with Litron's unfiltered lyophilized S9 was comparable to our

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standard AR20 S9 (unfiltered). While the recent results with the lyophilized S9 from Orgenics, Ltd. confirmed it's inability to activate 2R1 CSC, the additional results with a different source of S9 indicated that lyophilized S9 is suitable for evaluating CSC activity. It appears then there are some problems specific to the lyophilized S9 from Orgenics, Ltd.

V. VALIDATION OF A METHOD TO DETERMINE TRUE TOXICITIES IN THE IMC ASSAY (4)

In order to validate a method for the determination of agent-induced toxicity in the IMC assay, initial experiments were designed to find the minimum pre-incubation time necessary to prevent colony formation of 5E5 sensitive (S) cells inactivated with 6-thioguanine (6TG). This pre-incubation should also prevent metabolic cooperation. An additional experiment this month did not confirm previous findings (5). Since more consistent and acceptable results were obtained with pre-incubation at 72 hours, this time was used in an experiment to evaluate true toxicities in the IMC assay with catechol and 2R1 CSC. Results showed that the inclusion of 5E5 pretreated S cells in the test plates had no protective effect on the toxicity of catechol but some protective effect with 2R1 CSC. Plans are to further define the best pre-incubation time as well as determine agent-induced toxicity in the IMC assay.

VI. REFERENCES

1. Hackett, C. J. Jr. Notebook No. 8253, pp. 71-74.

2. Thompson, L. H. Notebook No. 8094, pp. 199-200.

3. Thompson, L. H. 6906 Monthly Progress Report. Monthly progress report 85-128; 1985, June 15.

4. Garcia, H. D. Notebook No. 8277, pp. 1-2.

5. McCoy, W. R. 6906 Monthly Progress Report. Monthly progress report 85-221; 1985, October 15.

Lisa H. Thompson

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CHARGE NUMBER: 6908

PROGRAM TITLE: SMOKE CONDENSATE STUDIES

PERIOD COVERED: NOVEMBER 1 - 30, 1985

PROJECT LEADER: R. D. KINSER

DATE OF REPORT: DECEMBER 2, 1985

I. NITROSAMINES

Nicotine-N'-oxide (NN'O) has been prepared for testing as a pyrosynthetic precursor of tobacco specific nitrosamines (TSNA) [1]. The product obtained appears to be a combination of cis and trans isomers. This product was applied to genetically low alkaloid burley filler (J5BUV) and the mainstream (MS) smoke from handmade cigarettes was analyzed for TSNA content. While NNK levels were greater than those measured for the untreated control, the primary difference attributable to the NN'O was a dramatic increase in MS NNN levels. Formation of NNN was not expected to be the preferred reaction path for NN'O, but these results indicate that NN'O may be an alkaloid progenitor of pyrosynthesized NNN.

Cigarettes made from filler treated with tocopheryl acetate (TA) were analyzed for MS TSNA content this month [1]. TSNA results obtained from three different TA-treated fillers have shown much more smoking-to-smoking variability than is usually observed. Whether this is due to uneven coating during the spraying process or an instability of the TA after application is not known, but studies of this potential inhibitor have been suspended.

Filler and MS TSNA values for new lots of genetically low alkaloid (LA) bright (Br) and burley (Bu) tobacco were determined [2]. Results from these analyses were compared with TSNA levels of previous lots of LA Br and Bu. Filler results for the two LA Bu samples were in reasonable agreement, but MS NNN and NNK deliveries were significantly greater for the newer lot. The newer LA Br sample had substantially lower filler TSNA levels, with some reduction in MS NNN and NAT. These results are consistent with the greater than expected MS TSNA delivery obtained for the recent machine-made low alkaloid blends.

Investigations of apparent artifactual formation of TSNA during smoke collection have been continued [3]. Increases in collection time and ascorbic acid levels both yielded increased TSNA levels. Addition of nornicotine to the bubbler traps resulted in increases in all three TSNA, suggesting that the observed artifacts may be due to transnitrosation reactions. Experiments to evaluate the possibility of transnitrosation in the bubbler trap environment are planned, in addition to tests of alternate inhibitors and a determination of the effect of trap pH.

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II. MAINSTREAM SMOKE STUDIES

Mainstream smoke was collected from nine different cigarette codes in twenty-eight smoking experiments for testing by members of Project 6906 [4]. New cigarette inventory procedures were implemented for cigarettes being transferred to off-site cold storage [4].

III. REFERENCES

1. Haut, S. A. Notebook 8167, p. 189.

2. Lambert, E. A. Notebook 8240, p. 39.

3. Morgan, W. R. Notebook 8218, p. 147.

4. Hellams, R. D. Notebook 8250, p. 65.

Robert D. Kinner

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R&D Support

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CHARGE NUMBER: 8101
PROGRAM TITLE: Cigarette Testing Services
SECTION LEADER: Joyce F. Stargardt
PERIOD COVERED: November, 1985

I. ANALYTICAL SUPPORT

A. ALKALOID CONTENT OF RECONSTITUTED MATERIALS SEPARATED FROM THE CIGARETTE BLEND (C. C. Bright)

An evaluation of the alkaloid levels for the reconstituted materials (recon) from Philip Morris blends indicated that the alkaloid level of the recon analyzed prior to blending is lower than the alkaloid content in recon separated from the total blend. Loew's Theaters, Inc. (Lorillard) was issued a patent in August, 1980 (#4,215,706) entitled "Nicotine Transfer Process", which documents this phenomenon using a strong acid or the salt of strong acid. Three experimental blends were used to confirm the patent claims: one containing 24% by weight RCB as the only recon in the blend, a second blend with 24% by weight RL-150B, and a third with 24% by weight RL-TC in the blend. The RCB sample had 6.5% diammonium phosphate applied, the RL-150B sample had 2.25% diammonium phosphate applied, and the RL-TC sample did not contain any diammonium phosphate. The RCB separated from the blend showed the highest alkaloid transfer from the lamina since it was treated with the highest concentration of diammonium phosphate. The RL-TC sample showed no increase in alkaloids, since it was not treated with diammonium phosphate.

B. TO INVESTIGATE THE UTILITY OF AN INFRAALYZER FOR THE DETERMINATION OF MENTHOL IN AFTERCUT SOLUTIONS (B. Kanipe)

Fourteen aftercut solutions were analyzed for menthol concentrations using the InfraAlyzer and the reference gas chromatographic method. The data generated by the I/A 400 compared favorably with g.c. results with the exception of two almost colorless solutions and one black solution. These color extremes were not in the original calibration set and were atypical of aftercut solutions. The InfraAlyzer is unable to accurately measure these menthol concentrations because of light penetration differences from the calibration set. While current results are favorable, at least twenty more afterecut solutions will be analyzed.

C. GLYCYRRHIZIC ACID CONTENT OF COMMERCIAL MENTHOL CIGARETTES (G. Carter)

Five commercial menthol brands were analyzed for glycyrrhizic acid, a flavor component found in licorice, in the tobacco filler. There was no glycyrrhizic acid detected in Kool 85 (B&W). Salem 85 and Vantage 85 (RJR) contained the highest quantities at 0.6 mg of glycyrrhizic acid per gram of tobacco filler. Newport 85 (Lorillard) had 0.3 mg/g and Benson & Hedges 100 (PM) contained 0.4 mg/g. These menthol brands were analyzed in order to establish baseline levels of licorice used in competitive menthol cigarettes.

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D. MICROSCOPIC BLEND SEPARATION (N. Ryan & B. Kanipe)

Microscopic Blend Separation was performed on Winston Lights 80 (Box). During the routine examination it was noted that this product contained a light colored reconstituted sheet as well as the typical Reynolds dark sheet. This light colored sheet is only in the Winston Lights 80 (Box) cigarettes. This product was analyzed for humectants and contained 1.1% triethylene glycol (TEG). This is atypical of Reynolds brands.

E. VARIABLE PUFF SMOKING MACHINE (B. Mait & B. Joyner)

The variable puff smoking machine is being used routinely to generate TPM and puff count data. The shut off valve for the gas cylinder connection to the variable puff smoking machine has been installed. This machine is now ready to be calibrated for CO delivery.

II. MARKET ACTIVITY (B. Mait & K. Sanderson)

Philip Morris will distribute Players Lights Menthol 85 and 100 cigarettes nationally in December, 1985. These cigarettes are packaged 25 cigarettes per pack and eight packs per carton for a total of 200 cigarettes. These cigarettes will sell for $1.50 less than regular price cigarettes.

Lorillard is nationally distributing True Gold 85 (Soft Pack) and True Gold 100 (Soft Pack and Box). The 85 mm cigarette delivers 11 mg tar and has cork tipping paper. The 100 mm versions deliver 12 mg tar and have white tipping paper.

III. PRESENTATION AND REPORTS

A. Memo to Mr. J. E., Wickham from W. A., Early, "The future Computer Needs of the Cigarette Testing Services Division", November 4, 1985.

B. D. R. Watson, "Borgwaldt Loose Ends Method", Method Number C-24, Analytical Division Methods Manual, November 20, 1985.

C. C. C. Bright to Mr. J., E. Wickham, "Alkaloid Content of Reconstituted Materials Separated from the Cigarette Blend", November 25, 1985, and presentation of November 26, 1985.

D. Memo from Becky Kanipe to Mr. J. E. Wickham, "Humectant and Plasticizer Levels of Commercial Cigarettes - 1985", November 21, 1985.

E. Memo from Becky Kanipe to Mr. J. E., Wickham, "Humectants in Winston Lights 80 (Box)", November 21, 1985.

JFS/bb

Joyce F. Stargardt acb

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